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Antiplaque effects and mode of action of a combination of zinc citrate and a nonionic antimicrobial agent

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Abstract - The effect upon plaque growth of adding a nonionic antimicrobial agent, triclosan, to a dentifrice containing zinc citrate was established in short-term in vivo studies. Plaque regrowth was inhibited by brushing with dentifrices which contained either zinc citrate or triclosan. When both were combined in the same dentifrice, the inhibition of overnight plaque regrowth was significantly greater. In two 4-day non-brushing studies, the dentifrices containing both zinc citrate and triclosan were applied either undiluted by the use of a cap splint or as 23% suspensions in water. Both methods resulted in significant reductions in plaque accumulation, with the greater activity being observed for the undiluted application of the dentifrice. Analysis of results of the overnight plaque studies for individual teeth revealed that the two agents had a complementary inhibitory action on plaque regrowth, zinc citrate being more effective on existing plaque whereas triclosan inhibited plaque formation on clean surfaces. The dentifrice containing both agents was effective against both existing plaque and new plaque formation. It is concluded that the addition of triclosan to a dentifrice containing zinc citrate improves its antiplaque potential.

Key words: dental plaque; dentifrice; triclosan; zinc citrate.

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Toothbrushing with a dentifrice remains the most common form of oral hygiene (1, 2), although its effectiveness varies widely, depending on people's attitudes and brushing habits. Ineffective brushing allows plaque to accumulate at the gingival margins of teeth and to cause gingival inflammation (1, 3). Since daily mechanical removal of plaque by

brushing commonly fails to maintain good gingival health, additional plaque control by chemical antimicrobial agents has been proposed (1, 4-7).

Incorporation of a cationic antiplaque agent (i.e. chlorhexidine) in dentifrices has been unsuccessful because of incompatibility with some of the normal dentifrice ingredi-

ents (8). A recent study (9) has confirmed that a 0.8% chlorhexidine (CHX) dentifrice, although it exhibited some ability to reduce plaque, was less effective than a 1% CHX gel. Other recent studies have shown that metal salts such as stannous fluoride and zinc citrate retain their antiplaque activity when incorporated into dentifrices (10-14).

This paper describes our continuing studies of the effect on plaque of using combinations of a metal salt (zinc citrate) and an antimicrobial agent (triclosan) in dentifrices. Zinc citrate has known antiplaque activity and is compatible with the normal components of dentifrices. Triclosan is a nonionic, phenolic antimicrobial agent (2,4,4'-trichloro-2'-hydroxydiphenyl ether) with good activity *in vitro* against a wide range of Gram-positive and Gram-negative organisms (15) including *S. mutans*, *S. sanguis* and *Actinomyces*. The bioavailability of zinc and triclosan from a dentifrice has been recently demonstrated *in vitro* (16).

Two plaque studies are described. These are respectively studies of (1) the effect of the two active agents, singly or combined, on overnight plaque regrowth, (2) the inhibitory effect of the combined agents on plaque accumulation over 4 days.

Material and methods

Materials - The placebo dentifrice contained aluminium trihydrate as abrasive, sodium monofluorophosphate, and other toothpaste ingredients such as humectant, binder, surfactant, flavour. The test dentifrices contained, in addition, 0.5% zinc citrate trihydrate (Sturge, Birmingham, UK), 0.2% triclosan (Ciba Geigy, Basel, Switzerland) or a combination of 0.5% zinc citrate trihydrate and 0.2% triclosan (i.e. "dual active").

Subjects - The informed consent of volunteers, men and women, aged 18 to 35 yr, was obtained prior to their participation in the studies. Persons who had acute infections or severe periodontitis or who were undergoing, or had recently completed, antibiotic or other antimicrobial drug therapy were specifically excluded. Volunteers exhibiting

periodontal pocketing (> 3.5 mm), extensive recession or untreated decay were also rejected. All studies were conducted double blind, and employed a cross-over design in which the dentifrices were used in random order.

Study Series 1 - Overnight plaque regrowth studies - This series consisted of two studies in which overnight plaque regrowth was assessed using the protocol suggested by HARRAP (17). Plaque assessment in both studies was made by the same examiner. In the first study, plaque regrowth assessments were made on 15 subjects after they had brushed their teeth with a placebo, or with a dentifrice containing either 0.2% triclosan alone or the above combination of both agents. In the second study using 23 subjects, plaque regrowths were compared after brushing with placebo, or with dentifrice containing either 0.5% zinc citrate or the combination of both agents.

The data were analysed using the paired *t*-test.

Study Series 2 - Four-day studies - The effectiveness with which the dentifrice containing both agents prevented plaque accumulation was compared with that of the placebo by using two protocols over a period of 4 days during which subjects abstained from brushing their teeth. In both studies the teeth were scaled on day 0, and to avoid carry-over effects 9 days were allowed between testing of dentifrices. In the first study 12 volunteers rinsed twice daily for 4 days with 13 g of 23% suspension of the test or placebo dentifrice. Plaque on the buccal surfaces of the teeth was then scored using the Extrinsic Stain Index (18, 19).

In the second study cap splints were prepared (10) for 10 volunteers. The splints were kept *in situ* twice daily for 2 min, each splint containing approximately 1.5 g of the placebo or test dentifrice. Plaque formation was enhanced by six daily oral rinses with 10 ml of 15% sucrose solution. After 4 days the plaque was assessed using the Plaque Index (20). The data were analysed using the paired *t*-test.

Results

Study Series 1 - Plaque regrowth - The mean plaque growths in 16 h of oral hygiene abstinence are shown in Table 1. The regrowth of plaque after use of the placebo was similar for both studies in this series, even though

different participants had been used. Regrowth of plaque at the gingival margin gave mean GMPI values of between 23 and 25. In both studies the inclusion of antimicrobial ingredients, either individually or combined, significantly inhibited the regrowth of plaque at the gingival margin. When both zinc citrate and triclosan were present in the dentifrice the mean plaque regrowth was significantly less than it was after use of either single-agent dentifrice.

The data were subjected to tooth-by-tooth analysis in an attempt to discover the mode of action of the two antimicrobial agents. Between 50 and 70% of the teeth were plaque-free immediately following toothbrushing, while most of the remaining surfaces had GMPI scores associated with the gingival margin ranging from 5 to 50. The relationship between the mean amount of plaque growth on each tooth and its baseline score (initial plaque score) for each of the dentifrices is shown in Table 2. The 'placebo' and 'test' data from the two studies have been combined for the analysis (i.e. data for the two placebo or two test dentifrices respectively).

Three categories of initial plaque values were formed (nil, small, medium).

When the 0.5% zinc citrate dentifrice was used, plaque growth appeared dependent on the initial plaque status. The percent difference between the plaque growths after use of placebo and zinc citrate dentifrice was highest on surfaces bearing the highest amount of initial plaque. In contrast, the greatest reduction after use of the triclosan dentifrice occurred on initially clean surfaces. With increasing amounts of initial plaque, its effect on plaque regrowth diminished (Fig. 1).

The combined data from the use of the dual-active dentifrice (Table 2) revealed a plaque growth inhibition pattern consistent with those found for each of the active agents. On initially clean surfaces inhibition of plaque growth was approximately 33% (Fig. 1). On surfaces with the highest initial amounts of plaque, inhibition was 50% but it was smaller for the intermediate values of initial plaque.

Study Series 2 - Four-day studies - The results of the first study are shown in Table 3. The

Table 1
Effect of dentifrices containing triclosan (Study 1) or zinc citrate (Study 2) or combination of both agents on plaque growth

Study	Dentifrice	Mean plaque growth†	Standard error
1 (n = 15)	Placebo	24.7	2.8
	0.2% triclosan	19.7	3.1
	0.2% triclosan	15.3*	2.8
	+ 0.5% zinc citrate		
2 (n = 23)	Placebo	23.2	2.4
	0.5% zinc citrate	18.2	2.3
	0.5% zinc citrate	14.7	1.5
	+ 0.2% triclosan		

† Gingival margin plaque units.

* Difference between dual active dentifrice and the other two dentifrices statistically significant at $P < 0.05$.

Table 2

Influence of initial plaque level on activity of dentifrices containing triclosan, zinc citrate or combination of both

Initial plaque score†	Mean plaque growth† (with number of teeth)			
	Placebo	0.2% triclosan	0.5% zinc citrate	0.2% triclosan + 0.5% zinc citrate
Nil	23.0 (404)	16.8 (179)	18.8 (195)	15.4 (385)
Small (10-20)	21.2 (107)	18.6 (34)	17.3 (66)	15.1 (100)
Medium (>20)	26.0 (58)	28.2 (34)	17.8 (36)	12.3 (69)

† Gingival margin plaque units.

mean tooth coverage of accumulated plaque when the suspension of the placebo was used over the 4-day period was 29.4%. The area of plaque following use of the test dentifrice was 20.4%.

In the second study the dentifrice was applied undiluted via a capsplint. The average Plaque Index score when the placebo was used was 1.15. When the dual-active dentifrice was used, a mean Plaque Index score of 0.65 was recorded.

Discussion

The addition of triclosan to the base toothpaste formulation (placebo) produced a sta-

tistically significant 20% inhibition of overnight plaque regrowth. A similar level of inhibition was observed when the zinc salt was added to the base formulation, and this confirmed the results of previous studies of dentifrices containing this agent (13).

Combining the two agents in the same base dentifrice significantly improved its efficacy. The antiplaque activity of the dual-active dentifrice was slightly less than the combined activities of the two agents. This supports the view that the two agents do not adversely interact with each other in the dentifrice.

Some insight into the relative roles of the two active agents has been obtained by considering their activity with respect to the condition of the surfaces directly after application. In the plaque regrowth studies, the teeth were cleaned by brushing and were not necessarily totally plaque free. Tooth by tooth analysis reveals something of the effect of the dentifrice on surfaces with varying amounts of plaque. Zinc had a small effect on plaque regrowth on surfaces which had originally been clean, but it had a greater effect on surfaces initially associated with moderate amounts of plaque (13). This suggests that the major effect of zinc is to reduce the rate of bacterial proliferation in existing plaque. In contrast, triclosan had a greater effect on those surfaces which were plaque free after brushing, while its inhibition of plaque growth decreased with increasing amounts of existing plaque. This suggests that triclosan may be adsorbed to the tooth surface and either prevents bacterial

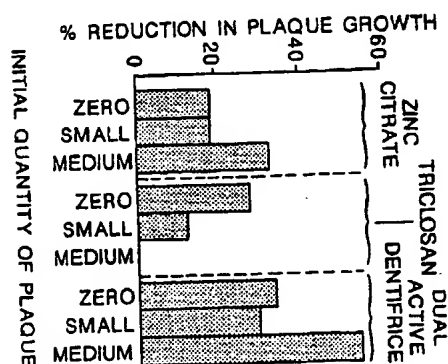


Fig. 1. Influence of tooth state after brushing on inhibition of plaque growth as result of using dentifrices containing active agents alone or in combination. Effect is shown as percentage difference between placebo and test dentifrice from data shown in Table 2.

Table 3

Effect of dentifrice containing both triclosan and zinc citrate on plaque accumulation during 4 days without toothbrushing

	Plaque scores (with standard error)		Plaque scoring
	Placebo	0.2% triclosan + 0.5% zinc citrate	
Rinse with 23% suspension	29.4 (2.9)	20.4 (2.7)	Area
Undiluted dentifrice from cap splint	1.15 (0.1)	0.64 (0.1)	Plaque index

adhesion or inhibits growth of the colonising bacteria, a mode of action shared by chlorhexidine (21). This complementary action by the two agents is advantageous for application in a dentifrice. The activity demonstrated in single application studies could be lost in multiple application regimens. This possibility was tested in the two regimens used during the 4-day studies. These regimens differed with respect to the mode of application and degree of dilution of the dentifrice. Both demonstrated that the test dentifrices reduced plaque accumulation compared to the placebo.

It is concluded that the inclusion of the antimicrobial agent triclosan and metal salt zinc citrate in a dentifrice in short-term studies produces a dentifrice which has enhanced antiplaque activity compared to either agent used separately and which is efficacious in 4-day studies.

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